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| 24728 | 7590 | 03/25/2011 | EXAMINER | |
| MORRIS MANNING MARTIN LLP | FERNANDEZ, SUSAN EMILY | | | |
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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|------------------------------|------------------------|---------------------|--|
| Office Action Summary | Application No. | Applicant(s) | |
| | 10/562,308 | KUHN ET AL. | |
| | Examiner | Art Unit | |
| | SUSAN E. FERNANDEZ | 1651 | |

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) Responsive to communication(s) filed on 20 December 2010.
 2a) This action is **FINAL**. 2b) This action is non-final.
 3) Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) Claim(s) 1-11 is/are pending in the application.
 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
 5) Claim(s) _____ is/are allowed.
 6) Claim(s) 1-11 is/are rejected.
 7) Claim(s) 11 is/are objected to.
 8) Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) The specification is objected to by the Examiner.
 10) The drawing(s) filed on 23 December 2005 is/are: a) accepted or b) objected to by the Examiner.
 Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
 Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
 11) The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
 a) All b) Some * c) None of:
 1. Certified copies of the priority documents have been received.
 2. Certified copies of the priority documents have been received in Application No. _____.
 3. Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413) |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | Paper No(s)/Mail Date. _____ . |
| 3) <input checked="" type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date <u>5/6/08, 8/21/08</u> . | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| | 6) <input type="checkbox"/> Other: _____ |

DETAILED ACTION

The amendment filed December 20, 2010, has been received and entered. Claims 1-11 are pending.

Election/Restrictions

Applicant's election with traverse of the species of ANNINE-6 as the voltage sensitive dye, in the reply filed on December 20, 2010, is acknowledged.

The traversal is on the ground that the voltage sensitizing dyes recited in claims 7, 8 and 11 are neither mutually exclusive nor patentably distinct from each other. This is not found persuasive because the criteria for an election of species requirement for a national stage application submitted under 35 U.S.C. 371 is whether the species lack unity of invention. The various voltage sensitive dyes of claims 7, 8 and 11 do not form a single general inventive concept under PCT Rule 13.1 since Zeck (PNAS. 2001. 98(18): 10457-10462. Listed on 5/6/08 IDS) teaches ANNINE-5 while not disclosing ANNINE-6, for instance (page 10458, second column, last paragraph).

Therefore, the voltage sensitizing dyes are not linked as to form a single general inventive concept. Though burden is not a requirement in determining unity of invention, it is noted that contrary to applicant's assertion, searching every species would indeed impose a serious burden on the Office as a search for one species would not necessarily encompass a search of another.

The requirement is still deemed proper and is therefore made FINAL.

Allowable Subject Matter

The elected species, the voltage-sensitive dye ANNINE-6, is considered to be free of the art when ANNINE-6 is defined as the chemical formula provided on Figure 1. Therefore, the examination has been extended to nonelected species **ANNINE-5 (formula (II) with m=1)**.

Pertinent art teaching ANNINE-6 is as follows (both listed on 5/6/08 IDS): Hubener (J. Phys. Chem. B. 2003. 107: 7896-7902); Kuhn (J. Phys. Chem. B. 2003. 107: 7903-7913). Both Hubener and Kuhn were published after the foreign priority date of the instant application and thus are not applicable as prior art.

Drawings

The drawings are objected to because the drawings are not numbered in consecutive Arabic numbers, starting from the first drawing to the last. The first four drawings are of tables each labeled as "Table" and a corresponding consecutive number starting from 1, while the following drawings are labeled as "Fig." starting off with "Fig. 1." As set forth in 37 CFR 1.84(u), the different views must be numbered in consecutive Arabic numerals, starting with 1, independent of the numbering of the sheets. View numbers must be preceded by the abbreviation "FIG."

Corrected drawing sheets in compliance with 37 CFR 1.121(d) are required in reply to the Office action to avoid abandonment of the application. Any amended replacement drawing sheet should include all of the figures appearing on the immediate prior version of the sheet, even if only one figure is being amended. The figure or figure number of an amended drawing should not be labeled as "amended." If a drawing figure is to be canceled, the appropriate figure must be removed from the replacement sheet, and where necessary, the remaining figures must be renumbered and appropriate changes made to the brief description of the several views of the drawings for consistency. Additional replacement sheets may be necessary to show the renumbering of the remaining figures. Each drawing sheet submitted after the filing date of an application must be labeled in the top margin as either "Replacement Sheet" or "New Sheet" pursuant to 37 CFR 1.121(d). If the changes are not accepted by the examiner, the applicant will be notified and informed of any required corrective action in the next Office action. The objection to the drawings will not be held in abeyance.

Specification

The disclosure is objected to because of the following informalities:

The chemical formulas on pages 4 and 9 show a residue "R₁" but this residue is recited as "R¹" in other portions of the specification (for instance, see page 5, lines 4-22 and page 9, line 20). This discrepancy in the chemical formulas and the description of said chemical formulas should be corrected such that only one form (R₁ or R¹) is consistently used.

The spacing of the lines of page 40 of the specification is such as to make reading difficult. New application paper(s) with lines 1½ or double spaced on good quality paper are required.

Also, the specification lacks a brief description of drawings as required by 37 CFR 1.74.

Furthermore, there are numerous instances throughout the specification that refer to figures that do not appear to correspond to the discussion. For example, the last two paragraphs on page 19 refer to Figure 6, though Figure 6 does not speak of electrochromic shifts and solvatochromic sensitivities. It appears that these passages should instead point to Figure 5. Other instances which appear to reference the wrong figure (not exhaustive): page 22, line 1; page 24, line 23; page 27, line 2.

The lengthy specification has not been checked to the extent necessary to determine the presence of all possible minor errors. Applicant's cooperation is requested in correcting any errors of which applicant may become aware in the specification.

Appropriate correction is required.

Claim Objections

Claim 11 is objected to because of the following informalities: The recitation "Voltage-sensitive dye" in line 1 should be replaced with "A voltage-sensitive dye." Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-10 are rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01.

The omitted steps are: (1) a step of adding the voltage-sensitive dye to a system in which a voltage change is to be detected, and (2) a positive step of measuring the fluorescence. Currently the only positive step required by the claimed method is irradiation of a voltage-sensitive dye with light. There is no step of actually adding the dye to a system (nor any system identified) in which the voltage change is to be measured, nor an active step of measuring the fluorescence. The omission of such a step makes the claimed method incomplete. Thus, claims 1-10 are rejected under 35 U.S.C. 112, second paragraph.

Claims 1-11 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 is indefinite since it is unclear how voltage changes are determined, and in what (i.e. in cells? in electrical systems?). The claim recite the steps of irradiating the voltage-sensitive dye with light and measuring the fluorescence [of the voltage-sensitive dye] caused by irradiation with light, but do not specify how these steps result in the measurement of voltage changes. For example, it is not clear if the voltage change is within the dye itself, or if the voltage change being measured is in another system (i.e. a biological cell or an electrical system)? As discussed above, the claims do not recite adding the dye to any system in which voltage change is to be measured, thus it is unclear what exactly is changing and what is being measured. Furthermore, it is unclear if the irradiation with light is to cause the voltage

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change, or if another stimuli is required. Thus, claims 1-10 are rejected under 35 U.S.C. 112, second paragraph. For examination purposes, the voltage change will be taken to correlate with a change in fluorescence caused by irradiation of the voltage-sensitive dye with light.

Claim 2 is further held as indefinite because it recites a broad limitation/range followed by a narrower limitation that falls within the broad limitation/range. A broad range or limitation together with a narrow range or limitation that falls within the broad range or limitation (in the same claim) is considered indefinite, since the resulting claim does not clearly set forth the metes and bounds of the patent protection desired. See MPEP § 2173.05(c). Note the explanation given by the Board of Patent Appeals and Interferences in *Ex parte Wu*, 10 USPQ2d 2031, 2033 (Bd. Pat. App. & Inter. 1989), as to where broad language is followed by "such as" and then narrow language. The Board stated that this can render a claim indefinite by raising a question or doubt as to whether the feature introduced by such language is (a) merely exemplary of the remainder of the claim, and therefore not required, or (b) a required feature of the claims. Note also, for example, the decisions of *Ex parte Steigewald*, 131 USPQ 74 (Bd. App. 1961); *Ex parte Hall*, 83 USPQ 38 (Bd. App. 1948); and *Ex parte Hasche*, 86 USPQ 481 (Bd. App. 1949). In the present instance, claim 2 recites the broad recitation absorption of $\leq 12\%$, and the claim also recites "in particular $\leq 8\%$ " and "preferably $\leq 2\%$ " which are the narrower statements of the range/limitation.

Claim 3 is indefinite since it is unclear what wavelengths would be considered "...in the longer wavelength range, related to the absorption maximum." First, the phrasing is confusing, as it is unclear how the "longer wavelength range" is related to the absorption maximum. Also, the claim does not define which wavelengths are considered in such a range, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention.

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Claims 5 and 6 are indefinite since it is unclear what is being used to determine voltage changes in cells and membranes, respectively. The confusion is created by the use of the term "it" in the first line of each of claims 5 and 6.

Regarding claim 6, the phrase "especially" renders the claim indefinite because it is unclear whether the limitations following the phrase are part of the claimed invention. See MPEP § 2173.05(d).

Claim 7 is indefinite since the recitation "...characterized in that, as voltage-sensitive dye, a compound of...is used..." uses confusing wording, as it appears to be grammatically incorrect. For examination purposes, claim 7 will be read as reciting a method according to claim 1 wherein a compound of formula (I) or formula (II) is used as the voltage-sensitive dye. The recitation of "R¹" renders the claim indefinite since the chemical structures of formulas (I) and (II) show "R₁". It is noted that the specification also demonstrates this inconsistency (pages 4 and 5). It is therefore unclear whether "R¹" indeed refers to "R₁" provided in the chemical structures. Finally, the recitation "which compounds..." in the last line renders the claim indefinite because the second line of the claim refers to only one compound.

Claim 8 is indefinite since it is unclear that the recitation "a voltage-sensitive dye" is in reference to the voltage-sensitive dye recited in claim 1. It is suggested that "a voltage-sensitive dye" be replaced with "the voltage-sensitive dye."

Claim 8 is also rendered indefinite by the recitation of the various ANNINE names (ANNINE-4, ANNINE-5, etc.) since they do not clearly define the structure of the dyes. It is noted that page 4, line 15 through page 5, line 21, provides a description of ANNINE-4, ANNINE-5, ANNINE-6, and ANNINE-7 (assumed to be last formula on page 4). Page 6, lines 4-7 defines ANNINE-8 as having formula (I) with n=3, and ANNINE-9 as having formula (II) with m=3. However, the metes and bounds are unclear given that page 5, lines 1-22 provides various preferences for each residue in formulas (I) and (II). Moreover, Figure 1 defines ANNINE-4, ANNINE-5, ANNINE-6, and ANNINE-7 differently from pages 4-5 in that

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the R and R₁ residues are defined specifically as butyl and (CH₂)₄SO₃⁻, respectively. The description of ANNINE-5 and ANNINE-6 on pages 4 and 5 is also inconsistent with the literature, as the formula of these two dyes in Kuhn (J. Phys. Chem. B. 2003. 107: 7903-7913. Listed on 5/6/08 IDS) matches with Figure 1 of the instant application (see Figure 1 on page 7903 of Kuhn). Clearly there is inconsistency as to the definitions of ANNINE-4, ANNINE-5, ANNINE-6, and ANNINE-7 in the specification, thus making it further unclear what compounds these names define.

Claim 9 is indefinite because it recites the Stark effect though it is unclear from claim 1 that the Stark effect occurs.

The recitation of “R¹” renders claim 11 indefinite since the chemical structures of formulas (I) and (II) show “R₁”. It is noted that the specification also demonstrates this inconsistency (pages 4 and 5). It is therefore unclear whether “R¹” indeed refers to “R₁” provided in the chemical structures. Also, the recitation “which compounds...” in the last line renders the claim indefinite since claim 11 does not refer to any compounds, and instead refers to a single voltage-sensitive dye. The term “compounds” lacks antecedent basis.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1, 3-9, and 11 rejected under 35 U.S.C. 102(b) as being anticipated by Zeck (PNAS. 2001. 98(18): 10457-10462. Listed on 5/6/08 IDS) in light of Kuhn (J. Phys. Chem. B. 2003. 107: 7903-7913. Listed on 5/6/08 IDS).

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Zeck teaches an experiment which obtains the response of a membrane-bound voltage-sensitive dye to electrical stimulation. See page 10458, last paragraph. Nerve cells were cultured on a silicon chip, and their membranes were stained with the amphiphilic hemicyanine dye 8-N,N-dibutylamino-2-azachrysene-butylsulfonate, also called Annine 5. The specification as filed for the instant application provides the structure of Annine 5 (ANNINE-5) (page 4, lines 20-25), which is a nonelected voltage-sensitive dye recited in instant claims 7 (formula (II) with m = 1), 8, and 11 (formula (II) with m = 2). Therefore, Zeck clearly anticipates instant claim 11, as Zeck teaches the voltage-sensitive dye ANNINE-5.

After the staining of the cell membranes with ANNINE-5, fluorescence pictures were taken with a confocal microscope at an illumination of 472.7 nm and a detection between 540-680 nm, wherein the cells were treated to AC voltage pulses (page 10458, last paragraph). Relative change of fluorescence in the attached region of the cell membrane was recorded with a photomultiplier (page 10458, last paragraph, through page 10459, first paragraph). Therefore, voltage changes by means of a voltage-sensitive dye were determined in cells and their membranes, wherein the voltage-sensitive dye ANNINE-5 was irradiated with light having a wavelength of 472.7 nm, and change (increase or decrease) of fluorescence caused by irradiation with light was measured.

The spectral data and analysis provided in the Kuhn reference can be used in determining the degree of absorption of ANNINE-5 for the experiment discussed in Zeck. Kuhn discloses one-dimensional spectra of fluorescence and voltage sensitivity for various voltage-sensitive dyes, including ANNINE-5 (Figure 6 on page 7909). The spectral maximum wavenumbers of excitation and emission are listed for ANNINE-5 on Table 1 of Kuhn as $23,155\text{ cm}^{-1}$ and $18,419\text{ cm}^{-1}$, respectively (page 7906). It is clear from the upper plot for ANNINE-5 in Figure 6 of Kuhn that these wavenumbers provide the absorption maximum (intensity = 1.0). As the inverse of wavelength is wavenumber, the wavelength of 472.7 nm taught in Zeck converts to a wavenumber of about $21,160\text{ cm}^{-1}$ [calculation: $\nu = 1/(472.7\text{ nm})^*$

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(10^9 nm/ 100 cm)]. It can be seen from the upper plot for ANNINE-5 in Figure 6 of Kuhn that a wavenumber of about 21,160 cm^{-1} results in an intensity of about 0.20. Therefore, Zeck teaches irradiating ANNINE-5 with light having a wavelength at which the dye has an absorption of $\leq 20\%$ of its absorption maximum (anticipating instant claims 1, 4-8). In turn, this means that Zeck also anticipates instant claim 3 (“longer wavelength, related to absorption” is indefinite and can encompass any wavelength).

In discussing the voltage sensitivity of ANNINE-5 in a neuron membrane, Kuhn indicates that it relies on the molecular Stark effect (page 7912, last paragraph). Therefore, the change of fluorescence radiation measured for ANNINE-5 in the Zeck reference is inherently caused by the Stark effect. Thus, instant claim 9 is also anticipated by Zeck.

A holding of anticipation is clearly required.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-11 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zeck in view of Denk (Science. 1990. 248(4951): 73-76. Listed on 5/6/08 IDS), and in light of Kuhn.

As discussed above, Zeck in light of Kuhn anticipates claims 1, 3-9, and 11. However, Zeck does not expressly disclose that two-photon excitation is effected when membrane-bound voltage sensitive dye was electrically stimulated and the relative change of fluorescence was measured.

Denk teaches that “molecular excitation by the simultaneous absorption of two photons provides intrinsic three-dimensional resolution in laser scanning fluorescence microscopy” (abstract). A two-photon excitation is effected by very high local instantaneous intensity provided by the tight focusing in a laser scanning microscope (LSM) combined with the temporal concentration of a femtosecond pulsed laser (page 73, last paragraph). A femtosecond pulsed laser results in an appreciable probability that a dye molecule absorbs two long wavelength photons simultaneously, thus combining their energy in order to reach its excited state (page 73, last paragraph). It was noted that two-photon excitation avoids "...the serious problems associated with chromatic aberration in the objective lens...and pinhole throughput losses...that plague confocal LSM" (page 75, first column, first paragraph). Two-photon excited fluorescence was expected to be advantageous over confocal LSM and area detector imaging since UV photodamage to biological cells and fluorophores would be confined to the volume from which information is collected (page 75, third column, third paragraph). Furthermore, it was found to not visibly cause morphological change to a living cell (page 75, third column, last paragraph). In experiments conducted by Denk for two-photo excitation, the colliding-pulse, mode-locked dye laser (CPM) (the femtosecond pulsed laser) emitting light at a wavelength of 630 nm was used (page 74, first column, first full paragraph).

At the time the invention was made, it would have been obvious to the person of ordinary skill in the art to have used substituted the confocal microscope with Ar laser taught for fluorescence

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measurements in Zeck with a femtosecond pulsed laser in combination with laser scanning microscope, when conducting the experiment of Zeck for determining the response of a membrane-bound voltage-sensitive dye to electrical stimulation. One of ordinary skill in the art would have been motivated to do this since it would have avoided the problems associated with confocal microscopy: chromatic aberration in the objective lens and pinhole throughput losses. Furthermore, this substitution would have been advantageous over the confocal microscopy technique used by Zeck since it would have confined UV photodamage to the neuron cells and ANNINE-5 dye to the volume analyzed, and there would have been the expectation of minimal morphological change to the neuron cells. In using a femtosecond pulsed laser such as that disclosed in Denk, there would have been a high probability that two-photon excitation is effected when practicing the Zeck experiment. Moreover, the femtosecond pulsed laser of Denk would illuminate the cells and ANNINE-5 with light of wavelength 630 nm. This wavelength corresponds to a wavenumber of about 15,900 cm⁻¹. From the upper plot for ANNINE-5 on Figure 6 of Kuhn (page 7909), this wavenumber corresponds to a relative intensity of emission of about 0.2. Therefore, if the particular wavelength disclosed in Deck is used, ANNINE-5 would have been irradiated with light having a wavelength at which ANNINE-5 has an absorption $\leq 20\%$. Thus, instant claim 10 is rendered obvious while meeting the limitations of instant claim 1.

Moreover, since it is obvious to have used any other femtosecond pulsed laser, it would have been obvious to have illuminated the cell sample of Zeck with light of various long wavelengths. As such, the wavenumbers would have resulted in the lower range provided on the upper plot for ANNINE-5 on Figure 6 of Kuhn. It therefore follows that various other dye absorptions would have been obtained, including an absorption of $\leq 12\%$ the absorption maximum of ANNINE-5. Thus, instant claim 2 is rendered obvious.

A holding of obviousness is clearly required.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SUSAN E. FERNANDEZ whose telephone number is (571)272-3444. The examiner can normally be reached on Mon-Fri 9:30 am - 6:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mike Wityshyn can be reached on (571) 272-0926. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Allison M. Ford/
Primary Examiner, Art Unit 1651

SUSAN E FERNANDEZ
Examiner
Art Unit 1651

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